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Observations on the influence of culture conditions of the fungal feeding preferences of *Folsomia candida* (Collembola: Isotomidae)

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With 5 figures

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1. Introduction

Collembola have shown marked species preference for fungi in choice experiments carried out under laboratory conditions; the fungi having been cultured on a variety of artificial substrates. Malt extract agar has been used in several choice experiments (McMillan 1976; Visser & Whittaker 1977), similarly Czapek-Dox (Addison & Parkinson 1978; Dash & Cragg 1972). Potato-dextrose agar has been used in a series of choice and food suitability studies (Harasymek & Sinha 1974; Mills & Sinha 1971). The use of pure cultures in selection and feeding studies may be of questionable validity as the conditions are artificial. Aspen leaf-litter has been employed in an attempt to relate selective feeding under laboratory conditions to observations made in the field (Visser & Whittaker 1977).

The palatability of a fungus as a food resource may be, in part, related to its species, with all its intrinsic physical and biochemical features, and to the nature of the culture conditions employed in its preparation. Further, the quality of the fungal resource can have important effects on the dynamics of collembolan populations, for, as shown by Booth & Anderson (1979), there is a correlation between the fecundity of the collembolan *Folsomia candida* (Willem) and the level of nitrogen available to the fungus during its culture.

As part of an integrated study of the interactions between soil fungi and Collembola in both artificial and natural systems the present series of investigations of the influence of culture regimes and nutrients on feeding preference by Collembola were performed.

2. Materials and methods

2.1. Collembola

Stock cultures of the Collembola *Folsomia candida* were maintained in sandwich boxes containing a base of plaster of Paris and charcoal, adapted from Goto (1960). Humidity was maintained within the chambers by regularly moistening with distilled water. The Collembola were routinely fed with commercially available Baker's yeast that was periodically checked and removed if contaminated.

Adult individuals for study were selected by carefully sifting cultures through a 300 μm sieve. Those retained were transferred to clean chambers and left overnight without food. Only those appearing healthy and active were subsequently selected and released, in groups of fifty, into the centre of the prepared Petri dishes.

2.2. Fungi

Stock cultures of *Mucor plumbeus* (Mich. *ex* Fr.), *Mucor ramannianus* (Mich. *ex* Fr.), and *Cladosporium herbarum* (Link *ex* Fr.) were maintained on 2% malt extract agar slopes. These fungi had been shown to be readily palatable and frequently highly favoured in choice experiments (Addison & Parkinson 1978; McMillan 1976; Mills & Sinha 1971; Singh 1969).

Some weeks prior to test culturing, plugs of each fungus were transferred to 1% water-agar plates to deplete their reserves of nutrients stored in the hyphae. Plugs from the periphery of colonies on these plates were subsequently used to inoculate the experimental cultures which were then incubated at 25 °C.

2.3. Experimental procedures

2.3.1. Evidence of preference

Malt-extract agar (2% MEA) was prepared from commercially available material. The soil-extract agar (SEA) was prepared by passing freshly collected grassland soil through a coarse sieve, added to distilled water plus agar, and then autoclaved for 30 minutes. The leaf-extract agar (LEA) was similarly prepared after washing and macerating the freshly collected oak (*Q. robur* L.) leaf-litter. Agar plugs were cut from the cultures and let into the water-agar substrate of the choice Petri dishes using a sterile cork borer. A water-agar substrate was found to maintain sufficient humidity, fungal viability, and the integrity of the experimental surface.

2.3.2. Influence of nutrients and culture conditions

Experiments were performed using the defined liquid culture medium of Levi & Cowling (1969). The carbohydrate source was glucose, whilst the nitrogen was defined using asparagine monohydrate. This culture medium allowed the exact regulation of nitrogen available to the growing fungus; levels of 2, 20 and 200 mg l^{-1} were used. The liquid-cultured fungi, for presentation to the Collembola, were prepared by lifting the fungal mat clear of the solution, washing with sterile distilled water and cutting into pieces of similar size to the agar plugs used in the solid fraction. Both liquid and solid (added agar) regimes were employed in this series of investigations. In a later experiment, the agar was replaced by small glass beads to produce a solid substrate upon which *M. ramannianus* was cultivated.

2.3.3. Influence of culture age

The three fungi were cultured on MEA plates which were inoculated at weekly intervals for eight weeks and incubated at 25 °C. Agar plugs, for presentation to the Collembola, were taken equidistantly from the inoculating plug in each case.

All the cultures were examined by lightly pressing a piece of double sided sellotape, attached to a clean glass slide, onto the surface of the culture at a point coinciding with the area from which the experimental samples were removed. The tape was then stained with lacto-phenol cotton blue and examined microscopically.

2.4. Design

In an attempt to obviate the possible influence of pheromenal aggregations (Verhoef *et al.* 1977) on selection and the numbers feeding, the fungal diets were presented in groups on the experimental surface. The samples were distributed around the dishes randomly, left overnight at room temperature, and laid out at random, prior to the addition of the Collembola, to avoid any directional stimuli.

2.5. Monitoring

Collembola present on each of the fungal diets were checked hourly with a low power binocular microscope and the numbers feeding upon the diets recorded. Care was taken to ensure that the agar plugs were flush with the surface of the water-agar base to avoid any cryptic response by the Collembola under test.

2.6. Statistics

The hourly, replicate data were tested using analysis of variance (single and double classification) (Parker 1973). Subsequent comparisons between the sample means of each experiment were made using the fixed-range test (L.S.R.). This uses the multiplier Q instead of T, and reduces the probability of making a type 1 error per analysis to the probability level employed (P = 5%).

The counts of feeding Collembola were subsequently transformed to percentage data for graphical representation and comparison. Where percentages are scattered around 50% their distribution is often approximately normal, but in these experiments the percent data often fell around 30% and in some cases were greater than 70%. In these cases normality could not be assumed. The arcsin (angular) transformation was used to correct the distribution of percentages to one approximating to normal, allowing the calculation of mean and standard error values for the feeding responses.

The use of feeding animal counts in statistical analysis can be open to criticism because these statistics assume that the observations made over time are independent of one another, and also random. In fact, with hourly counts they may not be completely so, although there was a great deal of movement by the Collembola between samples during the tenure of monitoring. The results of the first series of experiments were, however, so marked that this criterion was not likely to negate the overall conclusions.

3. Results

3.1. Evidence of preference

The selection of fungi by feeding Collembola, grown on a variety of agars is shown in Figure 1. When cultured on MEA *M. plumbeus* was strongly favoured ($P < 0.001$) over each of the others, which received similar levels of feeding. With a change to the undefined leaf-extract agar, *M. ramannianus* was less preferred in preference to the others ($P < 0.01$). When cultured on soil-extract agar, this species was strongly favoured over *M. plumbeus*, which in turn attracted more *F. candida* than *Cladosporium herbarum* ($P < 0.001$).

3.2. Influence of culture regime

The three fungi were grown in liquid culture and presented as previously outlined. Analysis showed that there was a highly significant difference between the numbers of Collembola feeding on each sample ($P < 0.001$, Figure 2). *M. ramannianus* was subject to the heaviest feeding pressure, whilst *M. plumbeus* was not favoured. The fungi were again cultured, using agar to produce a solid substrate, and offered to the Collembola in a similar choice experiment. *Cladosporium herbarum* was now the most frequently chosen sample, with *M. plumbeus* again not being favoured ($P < 0.01$).

To test more fully the influence of culture conditions on collembolan selectivity, *M. ramannianus* was cultured in both liquid and solid regimes. The Collembola were able to select between both culture sources on the same Petri dish. Analysis of the counts showed that solid regime samples were markedly preferred to liquid ones ($P = 0.002$). This exercise was repeated using the small glass beads as the solid fraction removing any possible influence of nutrients present in the small amount of agar. A preference against liquid regime samples was, however, still evident ($P = 0.01$).

3.3. Influence of nitrogen concentrations in the culture media

The experiment using liquid cultured material showed similar species selection by the Collembola as previously found, but there was also a significant intra-specific selection to the levels of nitrogen available in culture ($P < 0.001$, Figure 3). With all three species of fungi

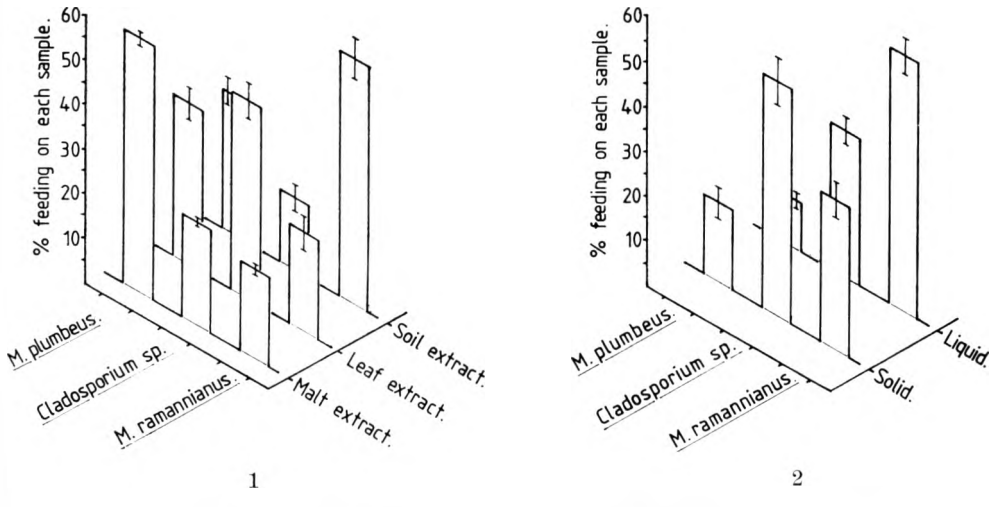
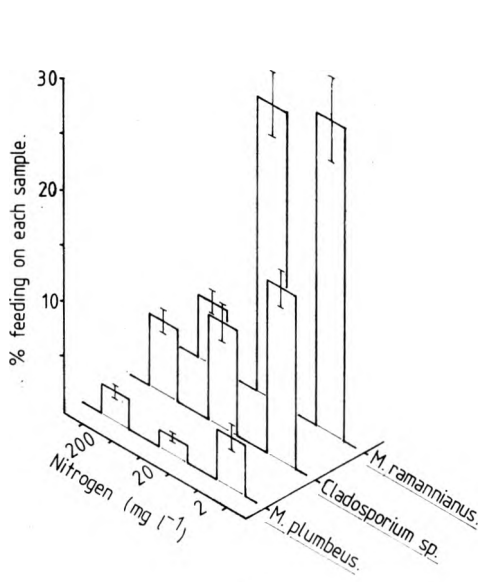


Fig. 1. The feeding response (as percent) of Collembola to three species of fungi grown on different extract agars; offered in three separate choice experiments. (Mean values ± 1 SE).

Fig. 2. The feeding response (as percent) of Collembola to three species of fungi grown on nutrient media. Results of four experiments; one series employing liquid conditions whilst the second had agar added to produce a solid growth substrate. (Mean values ± 1 SE).



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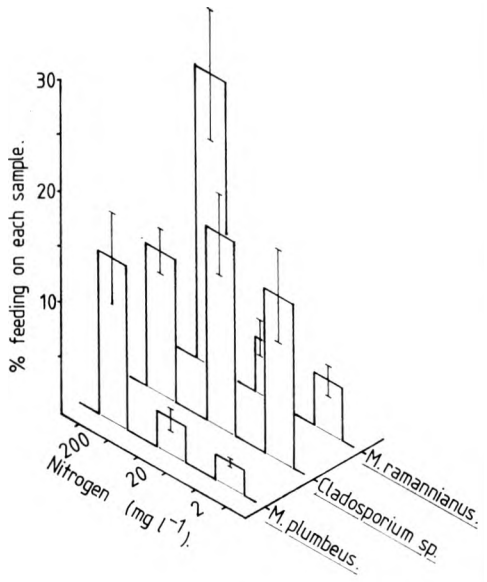

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Fig. 3. Inter- and intra-specific feeding preferences by Collembola to three species of fungi grown in liquid cultures containing three levels of nitrogen (as asparagine monohydrate) offered in two choice exeriments. (Mean values ± 1 SE).

Fig. 4. As figure 3, except a solid culture regime was employed. (Mean values ± 1 SE).

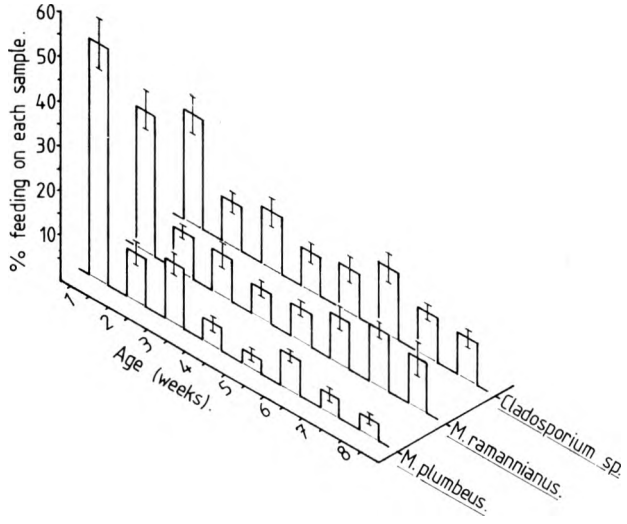

Fig. 5. Intra-specific responses (as percent) of Collembola to differing ages of fungal culture; offered in three separate choice experiments. (Mean values ± 1 SE).

under test the fungal diets grown in the highest levels of available nitrogen (200 mg l^{-1}) were not favoured. There was no discernable difference between the numbers feeding on the 20 and 2 mg l^{-1} samples of *M. ramannianus* ($P < 0.001$), a heavier selection of the lowest source material *M. plumbeus* ($P = 0.025$) and an increasing preference with a decreasing level of culture nitrogen with samples of *Cladosporium* ($P = 0.003$).

Feeding selection of solid regime samples also exhibited a significant response, although the trends were reversed ($P = 0.001$, Figure 4.). For the two *Mucor* spp. there was a switch in selection; the samples cultured in the highest level of nitrogen (200 mg l^{-1}) were strongly favoured (*M. ramannianus*, P = 001, *M. plumbeus*, $P = 0.025$). There was no significant difference between the levels of feeding on the three samples of *Cladosporium*.

3.4. Influence of culture age

As shown in Figure 5, the feeding Collembola strongly favoured the youngest cultures (< 1 week old) of all the three fungal species offered ($P < 0.001$ in all cases). In the case of *M. plumbeus* there was some evidence for a decline in preference with age, whilst in the other two there were no discernable differences in the low levels of feeding Collembola on the remaining ages.

Microscopical analysis of the samples showed that there were no discernible morphological differences between the youngest and all subsequent aged samples.

4. Discussion

The postulate raised by Tribe (1950) and Visser & Whittaker (1977), that any preference is a function of environmental conditions, is supported by this work. As shown in Figure 1 there was a different preference order for each of the extract agars employed. Using soil extract yielded a strong preference for *Mucor* species, whilst leaf extract showed the *Cladosporium* sp. to be favoured. This also shows the problem in making firm statements about collembolan preferences. Further, the change of the culture conditions had a profound effect on selectivity. Trinci (1969) demonstrated changes in the physical characteristics of fungi, when grown in liquid and on solid regimes. The fungal material fom solid cultures was strongly favoured in these investigations compared with that cultured in liquid conditions, although the available nutrients and other conditions were the same.

All these features are evidence for the existence of inter-specific selectivity. The investigations, however, also yielded information on intra-specific responses in collembolan feeding. The Collembola here avoided the fungal samples cultured in the highest level of nitrogen, preferring those from lower levels when grown in liquid conditions. Park (1976) demonstrated, for a wide variety of fungal species, that the physiological activity of a fungus (and therefore possibly its palatability to Collembola) was dependent upon the concentration of nitrogen in the growth medium. Further, Booth (1979) showed that the quality of a fungus, measured by its amino acid composition and concentration, improved with the increase of nitrogen in culture. A change to solid conditions produced a switch, particularly with the *Mucor* spp., to the highest level being strongly favoured and the, previously favoured, lower levels being almost ignored.

Another variable of the culture regime that influenced intra-specific selectivity was culture age (see also Mills & Sinha (1971) and Saichuae *et al.* (1972)). Sharma *et al.* (1977) showed that there was an increase in dry weight and total chitin content of several species of fungi with increasing culture age. The results presented show that there was a very marked preference for fungal material only a few days old and no major feeding on older material. Micro-arthropods, as a whole, however, are generally attributed the role in the decomposition processes of breaking biostasis through the browsing of senescent microbial tissues and the remobilisation of essential nutrients (Engleman 1961; Wallwork 1967). It is unlikely that Collembola will encounter material in a form similar to that used in these experiments. Further, the field community will be a heterogenous mixture of ages; however, this preference

indicates that although the Collembola will graze some of the senescent material, thereby releasing nutrients, this will be obviated by their preferential feeding on younger material.

Whatever the reasons behind inter- and intra-specific selectivity by Collembola, the interaction is complex, and influences the ubiquity of differing species both within and between habitats, as well as their roles in the dynamics of litter decomposition.

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Three species of soil and litter fungi were used in choice experiments to determine the effect of solid or liquid culture conditions, nitrogen concentrations of the media and the age of mycelium on selection by Collembola. Culture in litter, soil or malt extract media influenced inter-specific selection, while fungi grown on solid media were preferred to those cultured in liquid media. Intra-specific preferences were found for fungi grown under low nitrogen concentrations in liquid media; a pattern reversed in a similar series of experiments using fungi grown on solid media. Further evidence of intra-specific feeding by Collembola was found when they were presented with differing ages of culture; with material only a few days old being heavily browsed. It is concluded that nutrients and culture regimes are important in choice experiments.

Key words: Insecta, Apterygota, Collembola, Folsomia candida, feeding preferences, fungi, culture conditions.